The Association between Tumor Budding Peritumoral and Histologic Grade in Penile Squamous Cell Carcinoma

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Abstract

BACKGROUND: Squamous cell carcinoma (SCC) is the most common type of penile cancer. Inguinal lymph node metastatic is the most important factor for predicting survival in penile SCC patients and determining treatment options. Tumor budding is a biological phenomenon that has been described in malignancies and has both predictive and independent significant prognostic value. There is a lack of information about tumor budding in penile SCC.

AIM: This study aims to assess the association between tumor budding peritumoral with histological grade in penile SCC.

MATERIALS AND METHODS: Samples were taken from the paraffin blocks of patients diagnosed with penile SCC. The tumor budding peritumoral evaluation was classified as having fewer than five buds (low grade) and five or more buds (high grade) using H and E staining. The histological grade of penile SCC is assessed based on the WHO, and the ISUP grade scoring system evaluates based on nuclear pleomorphism with varying amounts of keratin production, which is divided into three grades; Grade I (well-differentiated carcinoma), Grade II (moderately differentiated carcinoma), and Grade III (poorly differentiated carcinoma). The association of tumor budding peritumoral with histopathological grade in penile SCC was analyzed statistically.

RESULTS: The mean age of SCC patients was 51.16 years old. The glans penis is the most prevalent site for a tumor, with stage IIIB being the most dominant stage. The majority of the samples were from lymph node metastases. Most of peritumoral budding tumors (60.7%) had high-grade budding. p = 0.002 was obtained from the Chi-square test, which showed that there is an association between tumor budding peritumoral with histopathological grade.

CONCLUSIONS: There is a significant association between tumor budding peritumoral with histopathological grade.

Introduction

Squamous cell carcinoma (SCC) is the most common type of cancer of the penile. Penile SCC is inevitably caused by the epithelium of the inner mucosal layer glans penile, coronal sulcus, and foreskin [1], [2]. Classification of penile SCC has evolved from time to time. In the 1890s, Paget used "epithelioma" for all penile malignancies. In 1921, Ewing declared and illustrated penile SCC as spindle cell carcinoma. Ackerman and Kraus (from the same institution) in 1949 and 1966 both used the terms "oral and genital verrucous carcinoma." Munoz and zur Hausen identified the association between human papillomavirus (HPV) and cervical and genital cancer. Attempts to classify invasive penile carcinoma subtypes based on their association with HPV infection were first made by Gregoire *et al.*, in 1995. They also introduced new terms such as "papillary carcinoma, NOS," and "mixed carcinoma." The current classification includes warty-basaloid, papillary-basaloid, and lymphoepithelioma-like carcinomas [1].

Penile cancer has a diverse epidemiology. The incidence is rare in developed nations such as the United States and Europe, depending on ethnicity and race. Meanwhile, higher incidences have been observed in South America, Southeast Asia, and Africa, with 1–2% of cases in men [1], [3]. Based on Global Cancer Statistics 2020, penile cancer ranked 33 out of 36 cancer types. Among 19,292,789 cases of cancer, penile cancer cases accounted for 36,068 cases (0.2%). Deaths from penile cancer are found in 13,211 (0.1%) of the 9,958,133 cancer deaths [4]. The incidence of penile SCC is increased with age, with the peak of age in the sixth decade, although rare in young men [3]. Penile cancer is associated with HPV infection and occurs in about one-third of all cases, which is the highest cause of penile cancer. Meanwhile, HIV and AIDS have no link to penile cancer [1], [3].

Penile cancer is Indonesia's 29th most prevalent cancer, accounting for 0.26% of new cases and 0.15% of deaths. In Indonesia, the overall incidence is 2.14/100,000 men [4]. Meanwhile, in Medan, according to data from the Anatomical Pathology Laboratory of Haji Adam Malik Hospital Medan, the prevalence of penile SCC from 2008 to 2011 was 15 cases [5].

Several penile cancer risk factors that are related, including phimosis, with an odds ratio of 11–16

compared with no phimosis, chronic penile inflammation, lichen sclerosis, psoralen and ultraviolet, smoking, HPV infection, condyloma acuminata, rural settings, poor socioeconomic status, and promiscuity [3].

Based on the WHO classification on penile tumors, penile SCC is the most common epithelial malignancy and is found in around 95% of cases. Basal cell carcinoma, penile sarcoma, melanoma, lymphoma, and metastatic disease are the following cancers that may occur. The SCC of the penile glands spreads lymphogenously to regional lymph nodes [1], [6].

A higher grade of tumor is associated with a metastatic condition. This condition is also related to lymphovascular or perineural invasions. Depending on lymph node involvement, low-risk and high-risk were classified [7], [8].

Tumor budding was defined as a single or group of cells containing at least one cell on the outer edge of an invasive tumor. The appearance of finger-like projections was found in various malignancies that entered the surrounding tissue. Tumor budding cells detach from the main tumor and infiltrate the surrounding stroma during localized cancer growth, which is histologically tumor metastasis or invasion that becomes an initial tumor metastatis. In other types of cancer, there were independent prognostic markers which related to distant metastases and co-related with the prognosis of the disease [9], [10].

Tumor budding has been studied in various organ cancers. Tumor budding is a novel prognostic marker in colorectal cancer that can help doctors forecast the likelihood of a bad outcome. In gastric cancer research, high-grade tumor budding has been associated to tumor grading, tumor invasion, and lymph node metastasis [11]. In lung adenocarcinoma, high-grade tumor budding is related to a higher recurrence rate than low-grade tumor budding [12]. In oral SCC, high-grade tumor budding is associated to a decreased chance of survival [13]. In squamous esophageal cancer, high-grade tumor budding is associated with a worse survival rate than low-grade tumor budding. In breast cancer, tumor budding is related to adverse pathological factors such as tumor size and lymphovascular invasion [14].

A budding tumor is a histopathological appearance that is diagnosed using hematoxylin and eosin (H and E) staining throughout a regular histopathological examination. According to Okamura *et al*., immunohistochemical staining of tumors is not superior to H and E staining in determining the existence of lymph node metastases by assessing budding lymph nodes [15].

Research on tumor budding of penile SCC is still limited and needs to be conducted. This research aims to determine the histopathological characteristics of penile SCC and analyzes the relationship of tumor budding peritumoral with the histopathological grading of penile SCC.

Methods

Design and samples

This is an analytical cross-sectional study. From 2013 to 2020, histopathology of penile SCC data was acquired from the Anatomical Pathology Department at Haji Adam Malik Hospital Medan. Forms and medical records were used to collect clinical data from patients. Age, tumor size, stage, lymph node metastases, histopathological grade, and budding tumor are all characteristics of the sample under research. Based on the calculation of the minimum sample, at least 27 samples can show significant results.

The working procedure was obtained as follows:

(1). Collect clinical data of penile SCC patients from medical records, including slide numbers and/or paraffin blocks, at Haji Adam Malik Hospital Medan's Anatomical Pathology Unit; (2) Clinical data (age, tumor size, clinical stage, and lymph node metastases) are recorded; (3). Hematoxylin-eosin staining, paraffin block collecting, cutting, and slide staining (H and E). Re-cut representative slides and paraffin blocks will be used to meet the inclusion requirements. The research sample consists of representative slides with comprehensive clinical data. (4). The researcher evaluated the tumor's grading, peritumoral budding, and the existence of lymphovascular and perineural invasion (5).

Peritumoral budding was obtained by: (a) using a slight magnification (40×) and picking the field of view with the most buds, finding, and determining the region of invasive tumor accompanied by tumor buds outside of the invasive tumor edge; (b). The defined field of view featured invasive tumor cell components and budding tumors at 200× magnification (c). Counting the number of peritumorally sprouted tumors. (d).The results of the budding tumor count are separated into two categories: low-grade buds (<5 buds) and high-grade buds (5 or more buds). (e) (Figure 1). The obtained data will be collated and a descriptive test was used to assess sample characteristics and a Spearman and Pearson used as correlation analysis. The test was used to examine the association between tumor budding peritumoral with a histological grade of penile SCC [16], [17], [18].

Results

From 2013 to 2020, 63 samples of penile SCC were collected at Haji Adam Malik Hospital in Medan. Consecutive sampling was used to select samples, and 28 samples met the inclusion criteria. The characteristics of the research sample are shown in Table 1.

Table 1: Characteristic of patients

Sample characteristics	Total (n)	Percentage
Age (years)		
20–29	2	7.1
30–39	4	14.3
40–49	7	25.0
50–59	6	21.4
60–69	9	32.2
Tumor location		
Glans	11	39.3
Prepuce	1	3.6
Coronal sulcus	0	0
Corpus penis	1	3.6
Location>1	6	21.4
Data (−)	9	32.1
Clinical stage		
I	5	17.9
II	6	21.4
IIIA	5	17.9
IIIB	9	32.1
IV	3	10.7
Lymph node metastasis		
M (−)	11	39.3
M (+)	17	60.7
Grade		
I	5	17.85
II	15	53.58
III	8	28.57
Lymphovascular invasion		
LVI ((−)	12	42.9
LVI (+)	16	57.1
Perineural invasion		
PNI (−)	22	78.6
PNI (+)	6	21.4
Tumor budding peritumoral		
<5 buds (low-grade buds)	11	39.3
≥5 buds (high-grade buds)	17	60.7

Most of the penile SCC is found in the age range of 60–69 years old (32.2%), followed by the groups of 40–49 years old (25%) and 50–59 years old (21.4%). The youngest age was 26 years old and the oldest was 67 years old, with an average age of 51.16 years old. Based on tumor location, penile SCC located on the glans penile is found in 11 patients (39.3%), six patients in more than one location (21.4%), one patient (3.6%) in prepuce, and one patient in corpus (3.6%), while 9 (32.1%) samples did not have information on tumor location. Based on the clinical stage, there were nine samples in stage IIIB (32.1%), six samples in stage II (21.4%), five samples in stage I and IIIA (17.9%), and three samples (10.7%) in stage IV. Lymph node metastasis is concluded by inguinal lymph node palpation, which is found in 17 samples (60.7%) and absent in 11 samples (39.3%). Lymphovascular invasion was found in 16 cases (57.1%), and based on perineural invasion, there were six samples that were positive (21.4%). From the histopathology, high-grade buds were found in 17 cases (60.7%) and low-grade buds in 11 cases (39.3%) (Figure 2).

Table 2 shows an analysis of the association of tumor budding peritumoral with histopathological grade. Five samples (45.5%) with histopathological Grade I, six samples (54.5%) with histopathological Grade II, and no samples of histopathological Grade III were obtained.

Table 2: Association of tumor budding peritumoral with a histopathological grade of penile SCC

Tumor budding peritumoral	Histopathological grade						p-value
	I		II		III		
	n	%	n	%	n	%	
Low-grade buds	5	45.5	6	54.5	0	0	0.002
High-grade buds	0	0	9	52.9	8	47.1	
Total	5	17.9	15	53.6	8	28.5	

While on high-grade buds, nine samples (52.9%) were in histopathological Grade II, eight (47.1%) were in histopathological Grade III, and no samples were found in histopathological Grade I. A p = 0.002 was obtained from the Chi-square test, which showed that there is an association between tumor budding peritumoral with histopathological grade.

Discussion

Penile cancer incidence in Indonesia is still lacking information. Mostly, it was discovered in men in their fifth or sixth decades of life; it was rarely found in children. While this research found the youngest age for penile cancer to be 26 years old, according to Wang *et al.*, the average age of penile SCC patients is 64 years old, ranging from 27 to 86 years [19]. Penile SCC can develop at any age. A sedentary lifestyle, smoking, and other harmful habits are all instances of an unhealthy lifestyle contributing to developing cancer. Furthermore, most young people are unconcerned with their health and frequently neglect to maintain and monitor their health.

The majority of penile malignancies were discovered in the glans penis (39.3%), followed by more than one place (21.4%), where the tumors could be seen simultaneously in the radix, corpus, and/or glans penis, and the least in the prepuce (3.6%). However, nine samples were discovered in the medical record without any information on the tumor's location. The findings of this study are similar to those of earlier investigations. Greenberg stated in 2010 that most penile cancer is detected in the penile glans, followed by the coronal sulcus and the inner surface of the prepuce [20]. In 2010, Chaux *et al*. reported the most tumor locations in the glans penile [21]. However, contrary to what Lagace *et al*. stated in 2020, the majority of tumors were found in non-specific places (54%), followed by the glans penile (25%), prepuce (16%), corpus penile (3%), and multiple lesions (3%) (2%) [22]. Men who have not been circumcised are more likely to develop penile SCC. It first appears in uncircumcised men's prepuce and circumcised men's penile glans. Smegma in uncircumcised men was thought to have components that caused penile cancer, but research revealed that not the component but smegma irritates the penile skin and induces inflammation. Chronic inflammation increases the risk of cancer development.

A secretion is produced by the prepuce in the penile beneath. If the prepuce is not thoroughly cleaned, the risk of penile cancer increases. Circumcision (in neonates), HPV infection prevention through prophylactic vaccination or the use of condoms, phimosis prevention, treatment of chronic inflammatory diseases, psoralen restrictions on psoralen plus ultraviolet light
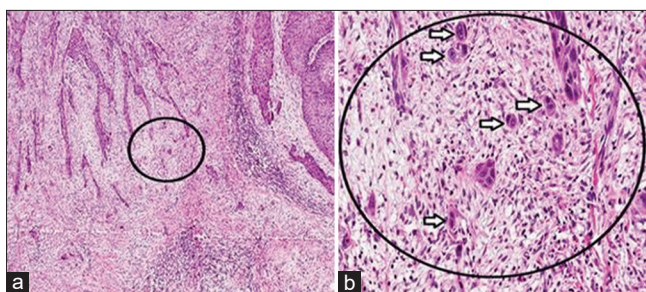
Figure 1: Tumor budding. (a). Tumor budding in front of invasive tumor (40×). (b). Tumor budding in large-field view (200×)

therapy, quitting smoking, and maintaining good hygiene are all preventive measures for penile SCC [1], [23], [24]. Penile SCC is typically curable if detected early [25]. The findings of this study suggest that changes in the penile's particular location are associated with patient survival. Tyson *et al.* published a study in 2012 with 2,515 cases of penile SCC for 34 years and found that cancers in the penile corpus and several locations had a higher

Figure 2: Histopathological findings. (a). Lymphovascular invasion (H and E, 200×), (b). Perineural invasion (H and E, 100×), (c). Low-grade buds (H and E, 200×), (d). High-grade buds (H and E, 200×), (e). Grade I (H and E, 100×), (f). Grade II (H and E, 100×), and (g). Grade II (H and E, 100×)

chance of death than tumors in the prepuce. Patients with cancers other than prepuce have a lower long-term survival rate. The findings could have substantial clinical consequences for determining the prognosis of penile SCC [26].

There were nine samples of penile SCC with inguinal lymph node enlargement or stage IIIB penile SCC included in this investigation. While lymph node involvement must be assessed through histological examination based on the TNM classification and the 8th edition of the AJCC clinical staging, it is essential to use the TNM classification and the 8th edition of the AJCC clinical staging (pathological classification of regional lymph nodes). According to the researcher, the medical record did not contain any of the above information. The presence and degree of metastases to the inguinal lymph nodes are critical prognostic factors determining penile SCC survival. The current staging technique for determining lymph node involvement in the regional lymph nodes is unreliable. Fifty percent of all clinically diagnosed inguinal lymph node metastases are not confirmed histopathologically [27].

In this investigation, 17 patients were found to have lymph node metastases. In this study, the majority of cases were at stage IIIB, which was consistent according to Salient *et al.*, inguinal lymph node metastases are the most important predictor of survival in patients with penile SCC. Lymph nodes are significant, because they determine the type of treatment required. When inguinal lymph nodes were involved, the 5-year survival rate dropped from 95.7% to 51.1% [28]. According to Hu *et al.*, the likelihood of lymph node metastasis increases as the stage of penile SCC progresses. When invasive pathological characteristics such as lymphovascular, corpora cavernosum, corpus spongiosum, urethral, and perineural invasion, bigger tumor size, and deeper tumor invasion are present, the likelihood of lymph node metastasis increases significantly [29].

Grade II penile SCC was discovered in 15 cases (53.58%). Because tumor grade predicts lymph node metastasis and correlates with overall survival. Nuclear pleomorphism, which ranges from well-differentiated to poorly-differentiated, and keratin production are used to identify tumor grade. Tumor cells proliferate in broadsheets with an uneven nesting pattern and a mildly reactive fibrous stroma on well-differentiated carcinoma (Grade I). Most tiny tumor nests with reactive stroma are moderately differentiated carcinomas (Grade II). The pearl is challenging to identify, and the development pattern is uneven and polymorphic in poorly differentiated cancers (Grade III). The stark distinction between Grade I and III aids in tumor grading. Grade II cases are indistinguishable from Grade I to III cases. Anaplastic cells are sufficient to classify the patient as having Grade III cancer. However, some sources suggest that at least 50% of anaplastic cells should be present. A significant degree
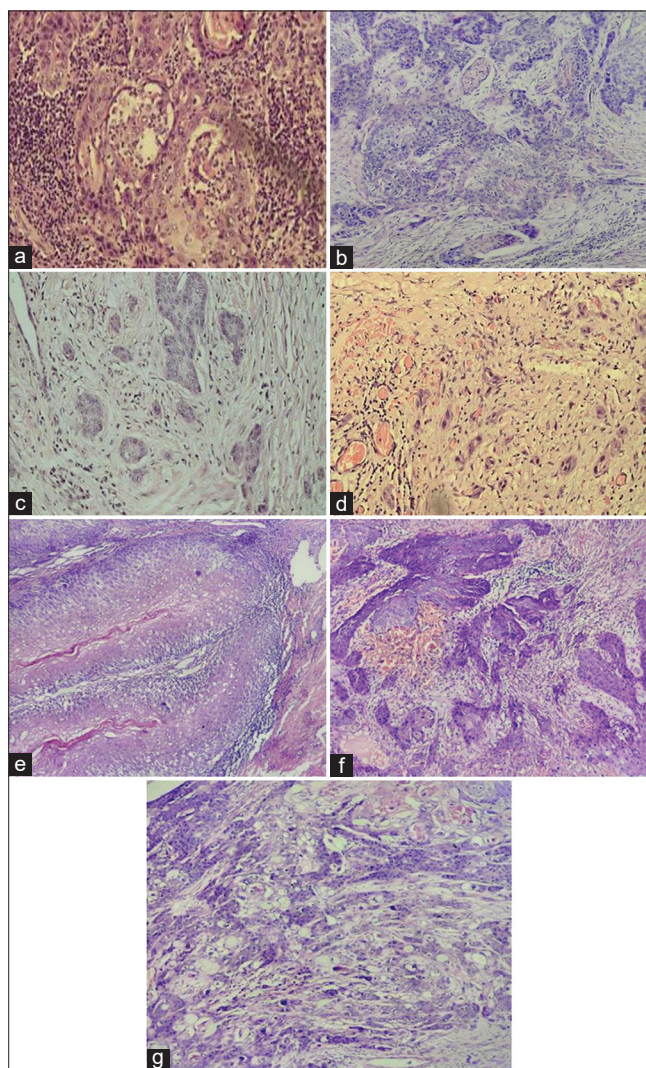
of nuclear pleomorphism in one broad field image should be considered while grading [1], [30]. The results of this study are identical to those of a previous study. In 2019, Sali *et al.* found that 59% of cases were Grade II (moderately differentiated cancer) [28]. Zheng *et al.* 57.3% of cases in 2020 were Grade II (moderately differentiated cancer) [31]. Nam *et al.* discovered that 82.7% of patients had carcinomas that were well-to-moderately differentiated [32].

Lymphovascular invasion was identified in 16 (57.1%) samples, and 12 (42.9%) samples were not observed. Only six samples of perineural invasion were detected out of 28. A higher risk of metastasis is linked to lymphovascular or perineural invasion. According to the research, lymphocytic invasion is thought to be a poor prognostic sign in a variety of cancers, including bladder cancer, prostate cancer, clear cell renal cell carcinoma, esophageal cancer, breast cancer, and lung cancer. Lymphocytic invasion is thought to be a bad sign in many cancers, including bladder, prostate, esophageal, breast, and lung cancers [33]. According to Li *et al.* in 2019, significant lymphovascular invasion will be followed by an increase in staging, grading, risk of lymph node metastasis, and risk of distant metastasis [34].

Table 2 demonstrates that high-grade buds account for most of the tumor blossoming peritumoral budding in 17 samples (60.7%), while low-grade buds account for 11 samples (39.3%). There is a link between tumor budding and epithelial-mesenchymal transitional (EMT). Due to EMT, epithelial cells become polarized and interact with the basement membrane through the basement surfaces. EMT can result in improved migratory capacity, invasion, resistance to apoptosis, and the formation of extracellular matrix components by undergoing biochemical changes that impact the phenotypic of mesenchymal cells [9]. EMT plays a key role in embryological development and tumor metastasis. One of the critical processes in EMT is the downregulation of E-Cadherin membrane proteins that are responsible for cell-to-cell adhesion. Through this process, tumor cells can escape from the tumor structure and promote carcinoma metastasis [35].

Tumor budding occurs both in the intratumor and the peritumor. Tumor budding has been utilized to predict poor prognosis in various carcinomas, including tongue, esophageal, lung SCC, pancreatic ductal adenocarcinoma, and colorectal adenocarcinoma. A growing tumor is linked to a higher risk of lymphogenic metastasis, a higher likelihood of recurrence, and a lower survival rate. A budding tumor is associated with decreased E-cadherin and increased vimentin expression in tumor cells and tumor buds on the invasive front. High- and low-grades might be assigned to a developing tumor. When counting budding tumors, spindle cells and cells with numerous nuclei indistinguishable from endothelial cells or fibroblasts are excluded. In contrast to H and E staining,

immunohistochemical staining such as pan-cytokeratin can distinguish tumor cells from fibroblasts and lymphocytes, which aids the detection of tumor cells at the invasive front [35].

A $p = 0.002$ was obtained from an analytical study of tumor budding peritumoral with a histopathological grade. It can be concluded that there is a significant relationship between tumor budding peritumoral with histopathological grade. Chatterjee *et al.*, in 2019, discovered a link between oral SCC budding tumors and histological grade (p-value = 0.0391) [36]. In 2019, Elseragy *et al.* also stated a relationship between budding tumors on oral tongue cancer with histopathological grade (p-value = 0.001) [37]. Budding tumors predict oral tongue SCC survival, according to Almangush *et al.* [17].

This study has some limitations. The higher metastasis risk in the presence of lymphovascular or perineural invasion cannot be proven since samples with enlarged lymph nodes, but no lymphovascular and/or perineural invasion were found in this study. In addition, lymphovascular and/or perineural invasion without lymph node enlargement was also found in this study. Another limitation of this study is that the researcher only used one stored paraffin block to assess lymphovascular and perineural invasion. According to the study findings, increased metastatic risk cannot be related to the occurrence of lymphovascular or perineural invasion.

Conclusions

There is a significant association between tumor budding peritumoral with histopathological grade.

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